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Quantitative fetal fibronectin for prediction of preterm birth in asymptomatic twin pregnancy

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Conflict of Interest Statement

Dr Hezelgrave-Elliott and Professor Shennan received financial assistance to provide educational talks on preterm birth from Hologic, USA. Preterm studies at Kings College London are funded by Tommys Charity but have minority funding and equipment support from Hologic, paid to the institution. The other authors do not report any conflicts of interest.

Abstract

Introduction

To evaluate cervicovaginal fluid quantitative fetal fibronectin, measured by a bedside analyser, to predict spontaneous preterm birth in twin pregnancy before 30 weeks of gestation.

Materials and Methods

In a prospective cohort study, we studied the accuracy of quantitative fetal fibronectin measured between 18 and 27^{+6} weeks of gestation in high risk asymptomatic women with twin pregnancies, to predict spontaneous preterm birth before 30 weeks of gestation. Predefined fetal fibronectin thresholds were greater than or equal to 10, greater than or equal to 50, and greater than or equal to 200 ng/ml. Predictive statistics were also calculated to evaluate accuracy of 'early' tests, performed between 18 and 21^{+6} weeks and 'standard' tests performed between 22^{+0} and 27^{+6} weeks of gestation in the same cohort. Subgroup analysis was performed according to cervical length measurement. In addition, we compared accuracy of prediction with quantitative fetal fibronectin measured during the standard test period in asymptomatic twin pregnancy with no additional risk factors, to twin pregnancies with one or more additional risk factors for spontaneous preterm birth.

Results

Of 130 eligible women identified with quantitative fetal fibronectin tests undertaken during the standard testing period, 9% delivered before 30 weeks of gestation. Quantitative fetal fibronectin was significantly related to outcome before 30/40 (ROC curves of 0.8 (CI 0.7 to

1). Early tests were not significantly predictive; ROC area 0.53 (CI 0.29 to 0.81) There was a trend towards better predictive accuracy when one or more additional risk factors for spontaneous preterm birth, or cervical length was considered.

Conclusions

Quantitative fetal fibronectin measured from 22 to 27^{+6} weeks of gestation accurately predicts spontaneous preterm birth at <30 weeks of gestation. Tests undertaken earlier are of limited value. Consideration of cervical length, or prior history in addition to qfFN strengthens prediction.

Keywords

Cervical length measurement, Fetal fibronectin, Premature birth, Pregnancy, Twin.

Abbreviations

CL Cervical Length

fFN fetal fibronectin

PPROM Preterm prelabour rupture of membranes

QfFN Quantitative fetal fibronectin

sPTB Spontaneous Preterm Birth

Key Message

Quantitative fetal fibronectin can be used to accurately predict preterm birth less than 30 weeks' gestation in twin pregnancy, in women who are asymptomatic of threatened preterm labour. Cervical length measurement, in addition, strengthens prediction.

Introduction

Twin pregnancies are increasing. There was a 60% increase in the UK between 1980 and 2009, with a further 20% rise between 2009 - 2015, mainly related to assisted reproductive technology and older maternal age at conception.[1] Preterm birth is the commonest morbidity associated with multiple pregnancy, 50% of twin pregnancies are preterm with 10% delivered at <32 weeks',[2] which puts the neonate at greater risk of respiratory distress syndrome, sepsis, cerebral palsy, cognitive defects and neonatal death. [3]

Twins are a powerful risk factor for preterm birth. There is little research on how established risk factors for singleton pregnancy, including prior spontaneous preterm birth (sPTB) or preterm prelabour rupture of membranes (PPROM), previous late miscarriage (16-23 $^{+6}$), and previous cervical surgery (Loop electrosurgical excision procedure, LEEP or cone biopsy), interact with twin pregnancy.

The pathophysiology of sPTB in multiple pregnancy is related to myometrial stretch triggering prostaglandin release, upregulation of oxytocin receptors with increased myometrial contractility, [4] which may be different from singleton pregnancy. Prediction of sPTB in twin pregnancy with fetal fibronectin (fFN) and cervical length measurement may therefore differ.

fFN is a glycoprotein located in the amniotic membranes. Its presence in cervicovaginal secretions suggests disruption of the membranes. Formerly a qualitative test with a positive/ negative result using a 50ng/ml threshold, it is now possible to establish the concentration of

fFN (qfFN) enabling more accurate prediction of preterm birth in symptomatic [5] and asymptomatic [6] singleton pregnancies. In asymptomatic singleton pregnancies the standard testing window is 22 to 27^{+6} weeks', although it has recently been shown that accurate prediction can be derived from 18 weeks'. [7] Qualitative fFN has been evaluated and found to be effective in symptomatic [8] and asymptomatic twin pregnancies, [9], [10] and can be used to help differentiate between women with a short cervix on ultrasonic cervical length (CL) measurement. [11]

Given increasing rates of multiple pregnancy, there remains an urgent need to optimise prediction of sPTB to avoid overtreatment of women with costly drugs and unnecessary admission to maternal and neonatal units. Therefore, this study aims to evaluate qfFN for prediction of sPTB in asymptomatic women with twin pregnancy, across the detectable range to define its potential value.

Materials and Methods

This was a secondary analysis of the EQUIPP Study (Evaluation of a Quantitative Instrument for Prediction of Preterm Birth, EQUIPP), a prospective masked observational study of 1448 high-risk asymptomatic women who underwent qfFN testing between 22^{+0}– 27^{+6} weeks' gestation at 5 UK teaching hospitals' . [6] All women with twin pregnancies regardless of risk factors for sPTB who had a qfFN sample taken between 18 and 27^{+6} weeks of gestation, and did not have symptoms of threatened preterm labour were eligible for inclusion in the analysis. Samples from women who reported prior sexual intercourse (within 24 hours) or frank bleeding visible on the swab were excluded as these are contraindications to using fetal fibronectin test. [12]

During speculum examination, a polyester swab was inserted into the posterior fornix of the vagina and rotated for 10 seconds to collect a sample of cervicovaginal fluid. The swab was immediately placed into the test buffer solution and analysed. One aliquot (200 microlitres) of the sample was analysed using the quantitative Rapid fFN 10Q analyser (Hologic, Marlborough, MA, USA) according to manufacturers' instructions. All clinicians received training to use the analysers. Based on our previous research [6] test thresholds of 10, 50 and 200ng/ml were predefined before the study data analysis. Following the swab, CL measurement (mm) by transvaginal ultrasonography was performed by trained staff and the shortest of three values used in the analysis. Clinicians were made aware of the categoric TLi $_{IQ}$ (Hologic, Marlborough, MA, USA) result (positive/negative), but both patient and clinician remained blinded to 10Q result until after delivery (a random result code generated by analyser), as per EQUIPP protocol. Women were managed in the clinic according to unit protocols, and were not blinded to positive/ negative fetal fibronectin result and CL measurement. Cerclage was performed at discretion of the clinicians given a lack of established evidence for this intervention.

Repeated fFN measurements were taken on each visit to Preterm Birth Clinic. For the purpose of this analysis, if there were more than two measurements, the first test obtained during an 'early' gestational age window (18^{+0} to 21^{+6}), and a 'standard' gestational age window ($22\text{-}27^{+6}$), was used. For women with tests obtained during 'early' *and* 'standard' gestation age windows, both tests were included in the analysis. If the sample was ineligible (e.g recent bleeding or sexual intercourse), it was excluded from analysis and the next available sample within the assessment window that fulfilled all criteria was used. If no appropriate sample was available the woman was excluded.

Pregnancy outcome details were collected from handheld notes, reviewed by trained research midwives, and entered onto the study database as the study progressed. Data entry was checked for inaccuracies contemporaneously by senior research midwives. Women were considered to have the outcome of interest (sPTB) if they had spontaneous onset of labour, or experienced PPROM with subsequent premature delivery.

Statistical Analyses

No formal power calculation was made but sample size was pragmatically based on analysing all of the available data on twins during the period of recruitment to the EQUIPP Study. Given the higher prevalence of PTB in twins, there was a reasonable expectation that meaningful predictive statistics could be calculated from the expected numbers (>100) recruited in the available time.

Statistical analysis was performed using Stata 11.2. Descriptive characteristics were presented for baseline demographics. QfFN results were grouped into four prespecified incremental categories (less than 10ng/mL, 10 to 49ng/mL, 50 to 199ng/mL, greater than or equal to 200ng/mL) and the corresponding sPTB rates calculated for each window. Non-parametric trend tests for qfFN at different levels were carried out for each predefined gestational endpoint, and ROC areas with 95% confidence intervals (CI) were calculated.

Predefined thresholds of 10ng/ml or greater, 50ng/ml or greater and 200ng/ml or greater were used to establish sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratios for prediction of sPTB by 'early' and 'standard' qfFN

tests before 30 completed weeks of gestation (primary outcome) and predefined outcomes of delivery before 34 and 37 completed weeks of gestation. ROC areas with 95% CI were calculated to determine overall predictive accuracy for 'early' tests.

The utility of CL measurement after stratification by qfFN was explored, as well as after stratification by the presence and absence of a short cervix (less than 25mm). This study is reported in accordance with Standards for the Reporting of Diagnostic Accuracy Studies. (Fig. 1).

Ethical Approval

The study was approved by the South East London Research Ethics Committee (REC no 10/H0806/68, London, United Kingdom). Written informed consent was obtained from all participants. Gestational age was confirmed with early Obstetric Ultrasound (11-14 weeks of gestation). Participant baseline demographics, obstetric history and risk factors were entered into an online secure study-specific database (www.medscinet.net/ptbstudies).

Results

A consecutive series of 5497 women meeting eligibility criteria were enrolled into the EQUIPP study. Of those women, 235 had a twin pregnancy, and 157 women had a valid qfFN test result in the pre-specified 'early' or 'standard' gestational age window. After applying exclusion criteria, analysis was performed on 92% (144/157) of women. Of those 144 women, 101 had 'early' tests and 130 women had 'standard' tests, with 87 having both early and standard tests. Women with iatrogenic delivery before the prespecified gestation endpoint under consideration were excluded from the analysis. (For 'early' tests: n=3 less

than 30 weeks of gestation, n=5 less than 34 weeks of gestation and n=26 less than 37 weeks of gestation and for 'standard' tests: n=4 less than 30 weeks of gestation, n=9 less than 34 weeks of gestation and n=37 less than 37 weeks of gestation) (See Fig. 1). Demographic and obstetric characteristics for the study participants are displayed in table 1. No adverse events were reported in relation to the qfFN test.

The sPTB rate for our primary outcome was 9% (11/126) at less than 30 weeks of gestation. It was 18% (22/121) at less than 34 weeks of gestation and 51% (47/93) at less than 37 weeks of gestation.

The proportion of women with sPTB according to fetal fibronectin concentration is shown in table 2. In this cohort, 90 (71%) were in the lowest category (less than 10 ng/mL). As qfFN increased so too did sPTB rate <30/40 for all gestational endpoints, increasing from 3/90 (3.3%) in the less than 10ng/mL category to 6/8 (75%) in the greater than 200ng/mL category for sPTB at less than 30 weeks of gestation. Non-parametric trend tests for fFN at different levels were significant ($p<0.01$) for our primary endpoint (30/40) but also our predefined gestational endpoints less than 34 and 37 weeks of gestation. ROC curves of 0.82 (CI 0.65 to 0.99); 0.74 (CI 0.63 to 0.86) and 0.67 (CI 0.59 to 0.76) for prediction of sPTB at less than 30, 34 and 37 weeks of gestation respectively, demonstrate overall predictive ability of qfFN regardless of threshold (see figure 2 and table 2).

The diagnostic accuracy of qfFN for predicting sPTB at less than 30 weeks of gestation with the use of prespecified thresholds of 10ng/mL, 50ng/mL and 200ng/mL is shown in table 3 for 'early' and 'standard' tests. For both tests, use of a higher threshold (>200ng/mL) considerably improved the specificity of the tests together with an improved PPV, whilst

retaining a high NPV. The ROC area for 'early' tests was 0.53 (CI 0.29 to 0.81) suggested no relation to gestational outcome unlike the 'standard' test where the ROC area of 0.82 (CI 0.65 to 0.99) was significant (table 2).

Of the cohort included in the analysis of qfFN with tests during the 'standard' gestational age window, 90% (117/130) had paired ultransonographic transvaginal CL measurements. Table 4 illustrates the proportion of women who delivered prematurely, stratified according to CL measurement and qfFN category. Short CL was related to outcome at all gestational endpoints.

For women with a short cervix (less than 25mm), sPTB rate at less than 30 weeks increased from 0% for those with low qfFN concentration less than 10ng/mL to 86% for those with qfFN concentration greater than or equal to 200ng/mL.

Conversely, in women with a high qfFN concentration greater than 200ng/mL, but a long cervix greater than 25mm PPV for sPTB was 0%. Although numbers were small, this suggests that combining qfFN and CL measurement strengthens prediction.

Of women with tests during the 'standard' gestational age window, 60% (78/130) had risk factors for sPTB in addition to twin pregnancy, including previous sPTB (n=27), previous PPROM (n=9), previous late miscarriage 16^{+0}-23^{+6} (n=12), previous cervical surgery (n=30). There was a trend towards improved prediction of sPTB for all gestational endpoints when at least one additional risk factor for sPTB was present. For our primary outcome of sPTB ROC areas were 0.87, CI 0.80 to 0.94 versus 0.55, CI 0.22 to 0.88 for prediction of sPTB less than 30 weeks of gestation for women with at least one additional risk factor compared to

twin pregnancy as the sole risk factor. But the difference (0.32, CI -0.07 to 0.71) was not significant.

Discussion

To our knowledge, this is the first study to demonstrate the diagnostic accuracy of differing concentrations of qfFN for prediction of sPTB in high-risk asymptomatic women with twin pregnancies. For example, for women with a qfFN concentration greater than or equal to 200ng/mL, 75% delivered less than 30 weeks of gestation compared to only 3% of those with a qfFN concentration less than 10ng/mL. A risk of sPTB of 3% is less than background risk in the general UK Obstetric population for twins. Therefore, clinicians can use the additional risk information afforded by quantifying fetal fibronectin to reassure women who are in fact low risk based on the results of the test, even in the presence of risk factors for sPTB in addition to twin pregnancy. Early qfFN tests (less than 22^{+0} weeks of gestation) do not have good prediction, contrary to singleton data, and should not be relied on until further research can confirm or refute this. [7]

Because the majority of tests (71%) are less than 10ng/mL, use of qfFN to determine discrete values provides a unique and new opportunity to provide reassurance to the majority of women who are theoretically at high risk; potentially reducing significant costs, both emotional and economic.

Quantitative fetal fibronectin test results also were synergistic with CL testing, more accurately discriminating those women with a short cervix destined to deliver early from

those who will not; of those women with a short cervix less than 25mm none with an fFN less than 10ng/mL delivered before 30 weeks of gestation compared to 86% of women with an fFN greater than 200ng/mL. This suggests value in combining these tests.

The association between increasing cervicovaginal fFN and progressive risk has been demonstrated in asymptomatic singleton pregnancy [6] and is biologically plausible because raised fFN represents disruption of the maternal-fetal interface. If degradation and release of fFN is the result of activation of matrix metalloproteinases release from immune cells following infection and/or inflammation, as has been hypothesized, [13] the more substantial the extent of infectious or inflammatory insult, the greater the release of fFN, corresponding with increased risk of delivery. These mechanisms may also be important in twins.
In twins, it has been suggested that additional factors contribute to increased risk of sPTB in twin pregnancy, related to uterine distension, and resultant myometrial stretch triggering prostaglandin release, upregulation of oxytocin receptors and increased myometrial contractility. Although non-significant, there was a trend towards better predictive accuracy when risk factors for sPTB in addition to twin pregnancy were present. A recent study by Easter et al, where women with a previous sPTB in addition to twin pregnancy compared to twin pregnancy as the sole risk factor had an increased likelihood of delivery at less than 35 weeks of gestation, (adjusted Odds Ratio 2.44, 95% CI1.28-4.66). [14] Therefore prediction in this group is important.

Conclusion

It has previously been shown that the combination of CL and fFN may be useful in predicting women with twin pregnancy who are asymptomatic but at high risk of sPTB. [11], [9], [15] However, these studies are based around qualitative, rather than quantitative fFN. The main

strength of this study is that it is the first to demonstrate the predictive accuracy of qfFN in asymptomatic twin pregnancy. This data has been incorporated into the QUIPP App (www.quipp.geneticdigital.co.uk) which combines qfFN, CL measurements and a woman's risk factors to calculate an individualised risk score to guide management, and now includes twins. Prophylactic interventions have been challenging in twins and further work is needed to establish linking prediction to intervention, and to evaluate qfFN for prediction of spontaneous preterm delivery in women with twin pregnancies with symptoms of threatened preterm labour.

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Tweetable Abstract:

Quantitative fetal fibronectin can accurately predict preterm birth in asymptomatic twin pregnancy. Cervical length measurement, in addition, strengthens prediction.

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Table 1. Demographic and Obstetric Characteristics of women with twin pregnancies tested for cervicovaginal fluid fetal fibronectin concentrations between 18 to 21^{+6} (n=101) and 22 to 27^{+6} weeks of gestation (n = 130)

	Value	
Characteristic	**Early 18-21^{+6} test (n=101)**	**Standard 22-27^{+6} week test (n=130)**
Age (y)	33.9 ± 5.2	34 ± 5.6
Ethnicity		
White	60 (59.4%)	81 (62.3%)
Black	27 (26.7%)	32 (24.6%)
Asian	8 (7.9%)	9 (6.9%)
Other	6 (5.9%)	8 (6.2%)
BMI (kg/m^2)	26.1 ± 5.8	25.9 ± 5.6
Smoking status		
Current	5 (5%)	6 (4.6%)
Ex- smoker (gave up before pregnancy)	24 (23.8%)	25 (19.2%)
Ex-smoker (gave up in pregnancy)	3 (3.0%)	4 (3.1%)
Never	69 (68.3%)	95 (73.1%)
Recurrent UTI	6 (5.9%)	8 (6.2%)
Domestic violence	3 (3.5%)	2 (1.7%)
Previous preterm birth	21 (20.8%)	27 (20.8%)
Previous PPROM	11 (10.9%)	9 (6.9%)
Previous late miscarriage	11 (10.9%)	12 (9.2%)
Previous cervical surgery	27 (26.7%)	30 (23.1%)
Cervical length <25mm this pregnancy	7 (6.9%)	14 (10.8%)
Cervical cerclage in current pregnancy	7 (6.9%)	10 (7.7%)

Table 2: Spontaneous Preterm Birth in Asymptomatic High-Risk Women according to Quantitative Cervicovaginal Fluid Fetal Fibronectin Categories for standard (22 to 27^{+6} weeks of gestation) test period.

qfFN, quantitative cervicovaginal fluid fetal fibronectin. Data are n (%) unless otherwise specififed. Non-parametric trend tests for fFN at different levels (<10, 10-49, 50-199, ≥200) for predicting delivery at <30, <34, <37 weeks of gestation are all significant, p<0.01. (*Women with iatrogenic deliveries before the gestation of analysis were excluded; see 'All' qfFN categories: n=4 at less than 30 weeks of gestation, n=9 at less than 34 weeks of gestation and n=37 at less than 37 weeks of gestation, which is reflected in the denominator changing at each gestational endpoint. Also see Figure 1 for iatrogenic exclusions).

		Spontaneous Preterm Birth (weeks of gestation)*		
qfFN Category (ng/ml)	**n (%)**	**Less than 30**	**Less than 34**	**Less than 37**
< 10	**90 (71.4)**	3/90 (3.3)	8/85 (9.4)	25/64 (39.1)
10-49	**21 (16.6)**	0/21 (0)	4/21 (19)	9/15 (60)
50-199	**7 (5.5)**	2/7 (28.6)	3/7 (42.9)	5/6 (83.3)
≥200	**8 (6.3)**	6/8 (75)	7/8 (87.5)	8/8 (100)
All	**126 (100)**	**11/126 (8.7)**	**22/121 (18.2)**	**47/93 (50.5)**
ROC Areas		**0.82 (0.65-0.99)**	**0.74 (0.63-0.86)**	**0.67 (0.59-0.76)**

Table 3: Prediction of Spontaneous Preterm Birth at less than 30 weeks of gestation According to Cervicovaginal Fluid Fetal Fibronectin concentration early (18 to 21^{+6}) and standard test (22 to 27^{+6}).

PPV, positive predictive value; NPV negative predictive value; LR, likelihood ratio. Data are % (95% confidence interval). The numbers of events are given in Table 2.

	Fetal Fibronectin Threshold (ng/Ml)					
Predictive variable	**10 or greater**		**50 or greater**		**200 or greater**	
	Early	**Standard**	**Early**	**Standard**	**Early**	**Standard**
Sensitivity	40 (12.2-73.8)	72.7 (39-94)	40 (12.2-73.8)	72.7 (39-94)	20.0 (2.5-55.6)	54.5 (23.4-83.3)
Specificity	75 (64.6-83.6)	75.7 (66.8-83.2)	88.6 (80.1-94.4)	93.9 (87.9-97.5)	94.3 (87.2-98.1)	98.3 (93.9-99.8)
PPV	15.4 (4.4-34.9)	22.2 (10.1-39.2)	28.6 (8.4-58.1)	53.3 (26.6-78.7)	28.6 (3.7-71)	75 (34.9-96.8)
NPV	91.7 (82.7-96.9)	96.7 (90.6-99.3)	92.9 (85.1-97.3)	97.3 (92.3-99.4)	91.2 (83.4-96.1)	95.8 (90.4-98.6)
Positive LR	1.6 (0.69-3.7)	3 (1.8-4.9)	3.5 (1.4-9.2)	12 (5.4-26.7)	3.5 (0.78-15.8)	31.4 (7.2-137.2)
Negative LR	0.8 (0.48-1.4)	0.36 (0.14-0.95)	0.68 (0.41-1.1)	0.29 (0.11-0.76)	0.85 (0.62-1.2)	0.46 (0.24-0.88

Table 4. Proportion of women with Spontaneous Preterm Birth when analysed according to cervical length measurement (above and below 25mm) and Quantitative Cervicovaginal Fluid Fetal Fibronectin category measured between 22 to 27^{+6} weeks of gestation. (*Women with iatrogenic deliveries were excluded, which is reflected in the denominator changing at each gestational endpoint).

		Spontaneous Preterm Birth (weeks of gestation)		
Cervical Length (mm) and qfFN Category (ng/Ml)	**n (%)**	**Less than 30**	**Less than 34**	**Less than 37**
Cervix 25mm or greater				
QfFN < 10	73 (80.2)	3/73 (4.1)	6/70 (8.6)	20/58 (34.5)
QfFN 10-199	17 (18.7)	0/17 (0)	1/17 (5.8)	7/13 (53.8)
QfFN ≥200	1 (1.1)	0/1 (0)	0/1 (0)	1/1 (100)
Total	**91 (100)**	**3/91 (3.3)**	**7/88 (8)**	**28/72 (38.9)**
Cervix less than 25mm				
QfFN less than 10	10 (38.5)	0/10 (0)	1/9 (11.1)	4/4 (100)
QfFN 10-199	9 (34.6)	2/9 (22)	6/9 (67)	6/7 (86)
QfFN ≥200	7 (26.9)	6/7 (85.7)	7/7 (100)	7/7 (100)
Total	**26 (100)**	**8/26 (30.8)**	**14/25 (56)**	**17/18 (94.4)**